ELECTROPHYSIOLOGIC CHANGES IN PATIENTS ADMITTED WITH NEUROTOXIC SNAKEBITE

Dissertation submitted in partial fulfillment of

requirements for

M.D. DEGREE IN GENERAL MEDICINE

BRANCH I

Of

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI, INDIA.

MADRAS MEDICAL COLLEGE,

CHENNAI 600003

MARCH 2010

DECLARATION

I solemnly declare that this dissertation entitled "***ELECTROPHYSIOLOGIC CHANGES IN PATIENTS ADMITTED WITH NEUROTOXIC SNAKEBITE*** " was done by me at Madras Medical College and Government General Hospital, during 2007-2010 under the guidance and supervision of **Prof. C RAJENDIRAN, M.D.** This dissertation is submitted to the TamilNadu Dr.M.G.R. Medical University towards the partial fulfillment of requirements for the award of M.D. Degree in General Medicine (Branch-I).

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Date:

ACKNOWLEDGEMENT

At the outset, I thank **Prof. J.MOHANASUNDARAM M.D.,** Dean, Madras Medical College and Government General Hospital, Chennai-3 for having permitted me to use hospital data for the study.

I am grateful to **Prof. C. RAJENDIRAN, M.D.,** Director and Head of Department, Institute of Internal Medicine, Madras Medical College and Government General Hospital, Chennai-3 for his support and painstaking efforts in scrutinizing the study.

My sincere wishes to **Prof. K. PADMA, M.D.,(Physiology)** Professor & HOD, Department of Physiology, Madras Medical College and Government General Hospital, Chennai-3 for her constant suggestions and guidance.

I would also like to thank my Assistant Professors **Dr. D. Basker, M.D.,** and **Dr. R. Muthuselvan, M.D.,** Madras Medical College and Government General Hospital, Chennai-3 for their support.

I am indebted to **Dr. V. Rajendran, Dr. T. Thangam, Dr. Ramesh, Dr. D. Thangam, Dr. Jeyakumar, Dr. G. Ravi,** Assistant Professors in Poison Control, Training & Research Centre, Govt. General Hospital, Chennai, Who guided me during the course of study.

My sincere wishes to all the patients who participated in the study.

Lastly, I thank all my Professional colleagues for their support and valuable criticisms.

CERTIFICATE

This is to certify that the dissertation entitled

" ***ELECTROPHYSIOLOGIC CHANGES IN PATIENTS ADMITTED WITH NEUROTOXIC SNAKEBITE*** " is a bonafide work done by **Dr. K**, **PRASHANTH** at Madras Medical College, Chennai in partial fulfillment of the university rules and regulations for award of M.D., Degree in General Medicine (Branch-I) under my guidance and supervision during the academic year 2007 - 2010.

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CONTENTS

Introduction

Envenoming resulting from snakebites is an important public health problem in many tropical and subtropical countries. Snakebites cause considerable morbidity and mortality worldwide. Venomous snakes are found throughout most of the world (including many oceans), except for a few islands, frozen environments, and high altitudes[18]. The highest burden exists in South Asia, Southeast Asia, and sub-Saharan Africa. Populations in these regions experience high morbidity and mortality because of poor access to health services, which are often suboptimal, and, in some instances, a scarcity of antivenom, which is the only specific treatment.

Recent estimates suggest that worldwide, venomous snakes cause "5.4 million bites, about 2.5 million envenomings and over 125,000 deaths annually"[20], "more than 3 million bites per year resulting in more than 150,000 deaths"[21], or "several million bites and envenomings annually with tens of thousands of deaths"[22]. Hospital mortality data are well known to underestimate overall mortality due to snakebites[25]

Of the 3,000 or so snake species that exist in the world, about 600 are venomous. In the country, approximately 95% of the mortality is attributable to bites by the cobra (Naja naja), Russell's viper (Daboia russelii), common krait (Bungarus caeruleus). The principal effects of envenomation with snake toxins are related to neurotoxicity, nephrotoxocity, myotoxicity, cardiotoxicity, coagulopathy, vascular endothelial damage and local reactions.

Snake venom is not a single toxin but a complex mixture of several components, including enzymes, polypeptide toxins, non-toxic proteins, carbohydrates, metals, lipids, free amino acids, nucleotides and bionic amines. Neurotoxins of snake venom seem to affect various sites of neuromuscular system Snake venom neurotoxins that bind to acetylcholine receptor sites on the motor endplate produce effects similar to those of curare and myasthenia gravis. Another group of neurotoxins with phospholipase A2 bind presynaptically, thereby depressing transmitter release, and are completely resistant to anticholinesterases. Respiratory muscle weakness is a potentially fatal manifestation of snake bite.

Because most snakebite victims are young[(19)], the economic impact of their disability is considerable. Despite the scale of its effects on populations, snakebite has not received the attention it deserves from national and international health authorities, and may therefore be appropriately categorized as a neglected tropical disease.

Aims and Objectives

- To define the snakes causing neurotoxicity,
- To analyse the demographic data of the patients admitted with neurotoxic snake bite
- To study the spectrum of neurological manifestations following envenomation,
- To identify the variations in the clinical presentation in patients with associated hemotoxicity and local cellulitis
- To study the pattern of electrophysiologic changes in the Nerve conduction studies and the correlation with outcome
- To study the disease course and outcome among patients admitted with neurotoxic snake bite

Review of Literature

Snake venoms can be broadly categorized into many types,:

1) **Hemotoxic Venoms**

These venoms attack the cardiovascular system, circulatory system and muscle tissues, thus directly leading to heart failures. The 'crotalus adamanteus', notoriously known as the western diamondback rattle snake, uses this deadly venom to make it's prey more pliable[(27)]. This venom causes the poisoning of blood and affects the blood clotting mechanism to such a grave extent, that the victim can die of internal bleeding. Usually, no pain nor any other symptoms can be observed for almost 1-3 hours (sometimes even 8). The effects of this venom can be seen as lethargy, headaches, nausea, vomiting, etc.. It is these venoms that usually cause excessive (and hideous) scarring, gangrene and permanent or temporary loss of motor skills.

2) **Neurotoxic Venoms**

These venoms go after the central nervous system and brain. They often result in respiratory paralysis . Their effect can range between mild seizures to death. Cobras, mambas, sea snakes, kraits and coral snakes are known to possess this venom. The king cobras (ophiophagus hannah) are the most infamous carriers of this venom. Neurotoxic venom is essentially nerve destroying. Hence, one can see speech and swallowing difficulties, drooling, difficulty in breathing, respiratory arrests, convulsions and sometimes even prolonged unconsciousness in

the victims. The milder symptoms are dizziness, tunnel vision, blurred vision and increased sweating. This venom causes a very fast degeneration of the synaptic nerves and this is the reason for the blockage of nerve impulses sent to and from the brain to the muscles[26].

3) **Cytotoxic Venoms**

This is a milder venom that generally causes only localized symptoms at the location of the bite. This is a cell destroying venom that destroys everything in it's path - blood vessels, cells and tissues. The symptoms of the invasion of this venom are generally seen around 10-15 minutes after the snake encounter . The results are generally localized pain accompanied by severe swelling and bleeding.. This venom causes blue/black spotting due to limited blood circulation[26].

4) **Myotoxic Venoms**

This venom is found in the 'bothrops moojeni' snakes, commonly known as the Brazillian lancehead snakes. This venom is known to cause muscular necrosis. Myotoxic venom contains peptides that destroy the muscle fiber proteins and result in myonecrosis (muscle destruction). In the very later stages of the spread of this venom, the muscle proteins enter the blood stream ultimately result in renal failure.

Hemotoxic Snake Bite

Hemostasis is a balance of two opposing forces: clot formation and dissolution. Appropriate clotting is essential at the site of a wound in order to maintain hemostasis, however clotting away from the site of a wound must be minimized in order to prevent life-threatening thrombotic events[27]. Regulation of clotting involves a series of zymogen conversions with extensive use of co-factors. Each stage involves the conversion of a precursor to an active form, which then is involved in the activation of a factor in the next stage of the cascade. The ultimate goal is the activation of the protease thrombin with the subsequent conversion of fibrinogen to fibrin for use in clot formation.

Effects Of Venoms Upon Hemostasis

Venoms often have profound effects upon blood coagulation, acting directly upon important clotting factors either by inappropriate activation or through prevention of activation. The same net effect (i.e. inability to stop bleeding) may be produced by dramatically different mechanisms allowing for the selective use of venom or venom components to address a specific deficiency in blood chemistry. As blood coagulation therapies or diagnostic tools, the most important snake venom components have been homeostatic or antithrombotic agents[27].

Russell's Viper Venom

The use of snake venoms as homeostatic agents is based upon early observations of the potent coagulative properties of snake venoms, the Russell's viper (*Daboia russelli*) in particular gaining early use as a treatment of hemophilia. The mechanism of action of *D. russelli* venom is in the activation of the factors V, X, IX, as well as Protein C [(27)]. While *D. russelli* venom is no longer used as a therapeutic agent, it still is used as a diagnostic agent to determine deficiencies in clotting factor X.

Prothrombin Activators

In addition to measurement of factor X by *D. russelli* venom, other blood chemistry research or diagnostic uses of snake venoms utilizes the enzymes affecting the coagulation cascade, such as prothrombin activators to measure factor V levels in patients' blood.

Prothrombin activators found in snake venoms can be divided into four groups:

Group I convert prothrombin to meizothrombin with activity insensitive to the presence of the non-enzymatic prothrombinase complex cofactors (CaCL2, factor V and phospholipids)

Group II and III activators are able to cleave both peptide bonds in prothrombin essential for the conversion of prothrombin to thrombin, the difference between the II and III being that the converting activity of II is strongly stimulated by phospholipids and factor Va in the presence of calcium while III is

only stimulated by CaCl2 and phospholipid [27]

Group IV activators are proteases that cleave prothrombin into non-active precursor forms of thrombin rather than converting prothrombin into the enzymatically active products.

Thrombin-Like Enzymes

Thrombin-like enzymes, such as those abundantly found in viper venoms, are used to determine the levels of fibrinogen in a plasma sample by converting fibrinogen to fibrin and the levels of fibrin subsequently measured against a baseline. One of these serine proteases, batroxobin from the Central American pit viper *Bothrops moojen*, [26] is quite useful in its ability, unlike circulating prothrombin, to form clots even in the presence of heparin. This allows for a patient's plasma to be monitored for levels of fibrinogen even whilst undergoing heparin therapy. Consequently, these studies concluded that batroxobin is quite useful not only as a diagnostic agent but also possibly as a direct therapeutic agent.

Neurotoxic Snake Bite

The nervous system functions by conducting an electrical signal or impulse along the length of the nerve and transmitting it across a junction (called the synaptic cleft) to another nerve or to a muscle fiber. When a nerve impulse reaches the terminus of the nerve, an influx of ions promotes the release of vesicles

containing a neurotransmitter such as acetylcholine, allowing this messenger molecule to diffuse across the synaptic cleft and bind to specific receptors.

Ion Channels

Many of the important binding sites of the nervous system, such as ion channels, were elucidated using bacterial neurotoxins. Channels for the ions Na+ and Ca2+ play crucial roles in the transmission of a nerve impulse and are found pre- and post-synaptically for sodium and presynaptically only for calcium channels.

Na+ channels are made up of four transmembrane loops with repeating binding sites. The neuronal sodium channels are important during the initial phase of action potential due to the voltage-sensitive production of an inward movement of Na+ and a rapid depolarization from the resting potential continuing to a slight positive overshoot[28]. In addition, Na+ channels play a major role in determining the excitability of central neurons as revealed through the bacterial toxins tetrodotoxin and saxitoxin being selective towards particular channel subtypes.

Thus, sodium channels are often described as being tetrodotoxin-sensitive or saxitoxin-sensitive if toxin binding interferes with the nerve impulse[29]. By preventing the agonist-induced conformational change in the receptor ion channel required for the influx of sodium that is essential for membrane depolarization, these toxins inhibit neurotransmitter action and induce paralysis.

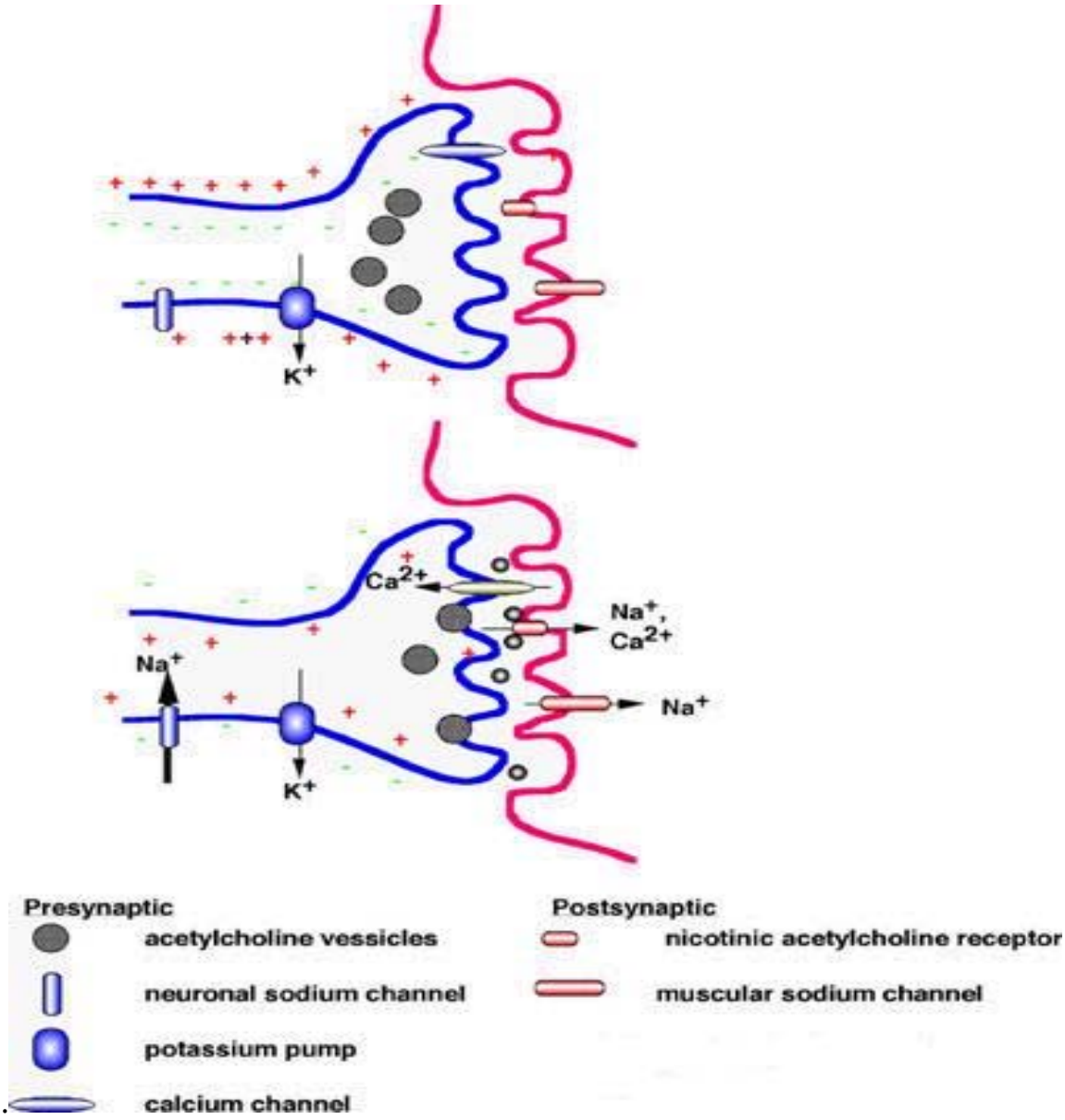


Fig 1. Basic structure of the neuromuscular junction showing the major channels and structures involved in nerve transmission.

Ca2+ channels, while not directly involved in the conductance of a nerve impulse, function to prolong the depolarization through the inward movement of Ca2+ thus causing the release of acetylcholine vesicles. Several calcium channel subtypes (L, N, P/Q, R and T) abound the nerve terminus, being differentiated through their sensitivity to different toxins. The P-type calcium channel, for example, was specifically characterized by its sensitivity towards the spider toxin omega-agatoxin[28][29].

Upon mobilization by the influx of Ca2+ through the calcium channels, the acetylcholine containing vesicles fuse with the membrane and exocytotically release their contents into the synaptic cleft and rapidly diffuse. Interference with the release of acetylcholine produces flaccid paralysis while increased release causes severe cramping of muscles. The toxins from the *Clostridium* bacteria are representative of the type that interferes with the release of acetylcholine. Botulism bacteria specifically causing flaccid paralysis through targeting the cholinergic motor nerve endings while the tetanus bacteria selectively cause spastic paralysis by targeting spinal neurons and causing an increase of acetylcholine release.

Acetylcholine Receptors (AchR)

Acetylcholine has two modes of action, a nicotine-like (nicotinic) or a muscarine-like (muscarinic) action, with the former blocked by curare and the later by atropine. Nicotinic acetylcholine receptors are found primarily at

neuromuscular junctions while muscarinic acetylcholine receptors are found primarily in the central nervous system. Functionally the two receptors are also different, nicotinic AChRs are ligand-gated ion channels while muscarinic AChRs are part of a larger class of G-protein coupled receptors[32]. This larger class utilizes the full-power of the intracellular secondary messenger system which involves an increase of intracellular Ca2+ .

Nicotinic Acetylcholine Receptors (nAChR)

Binding by two molecules of acetylcholine to the nicotinic AChR causes a conformational change resulting in the formation of an ion pore. This produces a rapid increase in cellular permeability of Na+ and Ca2+ ions, depolarization and excitation, resulting in muscular contraction[33]. Receptor subunits are either alpha (alpha2 - alpha9) or beta (beta2 - beta5) types, which leads to quite a number of potential combinations but the alpha-subunit is always present in two identical copies as these are the sites to which acetylcholine binds. The alpha-subunits also determine the binding sites through interaction with the other subunits. Neurotoxins targeting this site reversibly block the opening and prevent acetylcholine from forming a pore and allowing cations to pass through.

Neuronal nicotinic acetylcholine receptors (nnAChR) have been classified into two groups based on responses to the snake venom toxin alpha-bungarotoxin (BuTX)[35], being either alpha-BuTX-sensitive or insensitive. Alpha-BuTX-sensitive receptors are composed of alpha7, alpha8 and/or alpha9 subunits while

alpha-BuTX -insensitive receptors are composed of alpha2, alpha3, or alpha4 subunits with beta2, beta4 and/or alpha5 subunits.

Muscarinic Acetylcholine Receptors

Muscarinic receptors are found in the central nervous system synapses rather than at the neuromuscular junction, as is the case with nicotinic acetylcholine receptor specific toxins. Muscarinic receptors are involved in a large number of physiological functions including heart rate and force, contraction of smooth muscles and the release of neurotransmitters. Molecular cloning has determined five subtypes of muscarinic receptors, based on pharmacological activity they have been broken up into M1-M5. All five subtypes are found in the central nervous system while M1-M4 are also scattered widely through a myriad of tissues. M1, M3 and M5 receptors cause the activation of phospholipase C, generating two secondary messengers (IP3 and DAG) eventually leading to an intracellular increase of Ca2+, while M2 and M4 inhibit adenylate cyclase thus decreasing the production of the second messenger cAMP. Importantly, activation of the M2 receptor in the heart mediates the closing of calcium channels to reduce the force and rate of contraction. Ligand binding to the receptor causes a poorly understood conformational change that mediates the association with and activation of an intracellular G-protein. This G-protein converts GTP to GDP resulting in the disassociation of the activated G-protein allowing this enzyme to catalyze intracellular events.

Structure of snake neurotoxins

The venom from many snakes contain toxins that can bind to each type of receptor: the a-bungarotoxins act primarily on nicotinic AchRs at the neuromuscular junction, the k-bungarotoxins act primarily on nicotinic AchRs in neuronal tissue, and there are also muscarinic AchR-binding toxins[35]. These toxins show almost irreversible binding to the receptors, competitively inhibiting acetylcholine binding and, consequently, inhibiting the acetylcholine-induced electrical response. Both a- and k-bungarotoxins are three-finger toxins, their characteristic '3-finger' structure being determined by disulphide bonds[28][29]. This 3-finger fold allows for variation in structure, which can alter the function and selectivity of molecular targets. Evolutionary divergence has given rise to over 100 other post-synaptic a-neurotoxins found in *Elapidae* and *Hydrophiidae*, which may be related to the 3-finger proteins of vertebrates that play a significant role in cell-cell adhesion.

Other bungarotoxins

b-Bungarotoxin is much more lethal than either a- or k-bungarotoxin[34]. b-Bungarotoxin is a pre-synaptic toxin that acts on the (pre-synaptic) motor nerve terminals to block the release of acetylcholine. The action of b-bungarotoxin is complex. It has phospholipase A2 activity[30] which functions to hydrolyse phosphatidylcholine, in this case the phospholipids in the nerve membrane. Yet b-bungarotoxin displays both phospholipase-dependent and –independent activities[31]. b-Bungarotoxin is thought to bind to and block *Shaker*-type

potassium channels; the subsequent block of transmitter release is probably due to phospholipase A2-mediated destruction of the nerve terminal. Animals die as a consequence of respiratory failure.

Other toxins

Acetylcolinesterase (AchE) plays a key role in cholinergic nerve transmission, acting to breakdown acetylcholine to choline and acetate[32], which is important in controlling a receptor's response. Snake venom makes use of AChE to breakdown any neurotransmitter that might compete with a- or k-bungarotoxin for binding to AchRs. Venom AChE contains an additional exon over endogenous AChE, which generates a soluble form of the enzyme that is suitable for its venomous use[33].

Venom is an abundant source of nerve growth factor (NGF)[49], which induces neurite outgrowth. Venom NGF is often less potent than mammalian NGF, a family of neurotrophic factors that regulate the survival and differentiation of neurons[47]. Venom NGF acts as a low-potency agonist of TrkA-receptors, thereby competing for binding with endogenous NGF[48], affecting the survival of cholinergic neurons.

Competitive binding by the potent venoms of many animals produces interference of the binding of acetylcholine to the receptors resulting in flaccid paralysis.

Clinical Features

A proportion of patients bitten by venomous snakes (between <10% to >60%), depending on the species, will develop minimal or no signs of toxic symptoms (envenoming) despite having puncture marks which indicate that the snake's fangs have penetrated the skin.

Fear and effects of treatment, as well as the snake's venom, contribute to the symptoms and signs. Even patients who are not envenomed may feel flushed, dizzy and breathless, with constriction of the chest, palpitations, sweating and acroparaesthesiae[26]. Tight tourniquets may produce congested and ischaemic limbs; local incisions at the site of the bite may cause bleeding and sensory loss; and herbal medicines often induce vomiting.

The earliest symptoms directly attributable to the bite are local pain and bleeding from the fang punctures, followed by pain, tenderness, swelling and bruising extending up the limb, lymphangitis and tender enlargement of regional lymph nodes. Early syncope, vomiting, colic, diarrhoea, angio-oedema and wheezing may occur in patients bitten by European Vipera, *Daboia russelii*, *Bothrops* sp, Australian Elapids and *Atractaspis engaddensis.* [26]Nausea and vomiting are common symptoms of severe envenoming.

Types of bites

Elapidae (cobras, kraits, mambas, coral snakes and Australian venomous snakes)

Bites by kraits, mambas, coral snakes and some cobras (e.g., *Naja haje* and *N. nivea*) produce minimal local effects, whereas bites by African spitting cobras (*N. nigricollis, N. mossambica*, etc.) and Asian cobras (*N. naja, N. kaouthia, N. sumatrana*, etc.) cause tender local swelling which may be extensive, blistering and superficial necrosis.

Early symptoms of neurotoxicity before there are objective neurological signs include vomiting, “heaviness” of the eyelids, blurred vision, fasciculations, paraesthesiae around the mouth, hyperacusis, headache, dizziness, vertigo, hypersalivation, congested conjunctivae and “gooseflesh”. Paralysis starts as ptosis and external ophthalmoplegia appearing as early as 15 minutes after the bite, but sometimes delayed for ten hours or more. Later the face, palate, jaws, tongue, vocal cords, neck muscles and muscles of deglutition become progressively paralysed. Respiratory failure may be precipitated by upper airway obstruction at this stage, or later after paralysis of intercostal muscles, diaphragm and accessory muscles of respiration. Neurotoxic effects are completely reversible, either acutely in response to antivenom or anticholinesterases (e.g., following bites by Asian cobras, some Latin American coral snakes-*Micrurus,* and Australian death adders-*Acanthophis*) or they may wear off spontaneously in one to seven days.

Feature	Cobras	Kraits	Russells Viper	Saw Scaled Viper	Hump Nosed Viper
Local Pain/ Tissue Damage	**YES**	**NO**	**YES**	**YES**	**YES**
Ptosis/ Neurological Signs	**YES**	**YES**	**YES!**	**NO**	**NO**
Haemostatic abnormalities	**NO**	**NO!**	**YES**	**YES**	**YES**
Renal Complications	**NO**	**NO**	**YES**	**NO**	**YES**
Response to Neostigmine	**YES**	**NO?**	**NO?**	**NO**	**NO**
Response to ASV	**YES**	**YES**	**YES**	**YES**	**NO**

Table 1 Clinical Features of bites by various snake species

Envenoming by Australian snakes causes early vomiting, headache and syncopal attacks, neurotoxicity, haemostatic disturbances and, with some species, ECG changes, generalized rhabdomyolysis and kidney failure. Painful enlargement of regional lymph nodes suggests impending systemic envenoming, but local signs are usually absent or mild except after bites by *Pseudechis* sp.

Venom ophthalmia caused by "spitting" elapids

Patients "spat" at by spitting elapids experience intense pain in the eye, conjunctivitis, blepharospasm, palpebral oedema and leucorrhoea. Corneal erosions are detectable in more than half the patients spat at by *N. nigricollis*. Rarely, venom is absorbed into the anterior chamber, causing hypopyon and anterior uveitis. Secondary infection of corneal abrasions may lead to permanent blinding opacities or panophthalmitis.

Viperidae (vipers, adders, rattlesnakes, lance-headed vipers, moccasins and pit vipers)

Local envenoming is relatively severe. Swelling may become detectable within 15 minutes but is sometimes delayed for several hours. It spreads rapidly and may involve the whole limb and adjacent trunk. There is associated pain and tenderness in regional lymph nodes. Bruising, blistering and necrosis may appear during the next few days. Necrosis is particularly frequent and severe following bites by some rattlesnakes, lance-headed vipers (genus *Bothrops*), Asian pit vipers and African vipers (genera *Echis* and *Bitis*). When the envenomed tissue is contained in a tight fascial compartment such as the pulp space of the fingers or toes or the anterior tibial compartment, ischaemia may result. If there is no swelling two hours after a viper bite it is usually safe to assume that there has been no envenoming. However, fatal envenoming by a few species can occur in the

absence of local signs (e.g., *Crotalus durissus terrificus, C. scutulatus* and Burmese Russell's viper).

Blood pressure abnormalities are a consistent feature of envenoming by Viperidae. Persistent bleeding from fang puncture wounds, venepuncture or injection sites, other new and partially healed wounds and post partum, suggests that the blood is incoagulable. Spontaneous systemic haemorrhage is most often detected in the gums, but may also be seen as epistaxis, haematemesis, cutaneous ecchymoses, haemoptysis, subconjunctival, retroperitoneal and intracranial haemorrhages. Patients envenomed by the Burmese Russell's viper may bleed into the anterior pituitary gland (Sheehan's syndrome).

Hypotension and shock are common in patients bitten by some of the North American rattlesnakes (e.g., *C. adamanteus, C. atrox* and *C. scutulatus*), *Bothrops*, *Daboia* and *Vipera* species (e.g., *V. palaestinae* and *V. berus*). The central venous pressure is usually low and the pulse rate rapid, suggesting hypovolaemia, for which the usual cause is extravasation of fluid into the bitten limb. Patients envenomed by Burmese Russell's vipers show evidence of generally increased vascular permeability. Direct involvement of the heart muscle is suggested by an abnormal ECG or cardiac arrhythmia. Patients envenomed by some species of the genera *Vipera* and *Bothrops* may experience transient recurrent fainting attacks associated with features of an autopharmacological or anaphylactic reaction such as vomiting, sweating, colic, diarrhoea, shock and angio-oedema, appearing as early as five minutes or as late as many hours after the bite.

Renal (kidney) failure is the major cause of death in patients envenomed by Russell's vipers who may become oliguric within a few hours of the bite and have loin pain suggesting renal ischaemia. Renal failure is also a feature of envenoming by *Bothrops* species and *C. d. terrificus*.

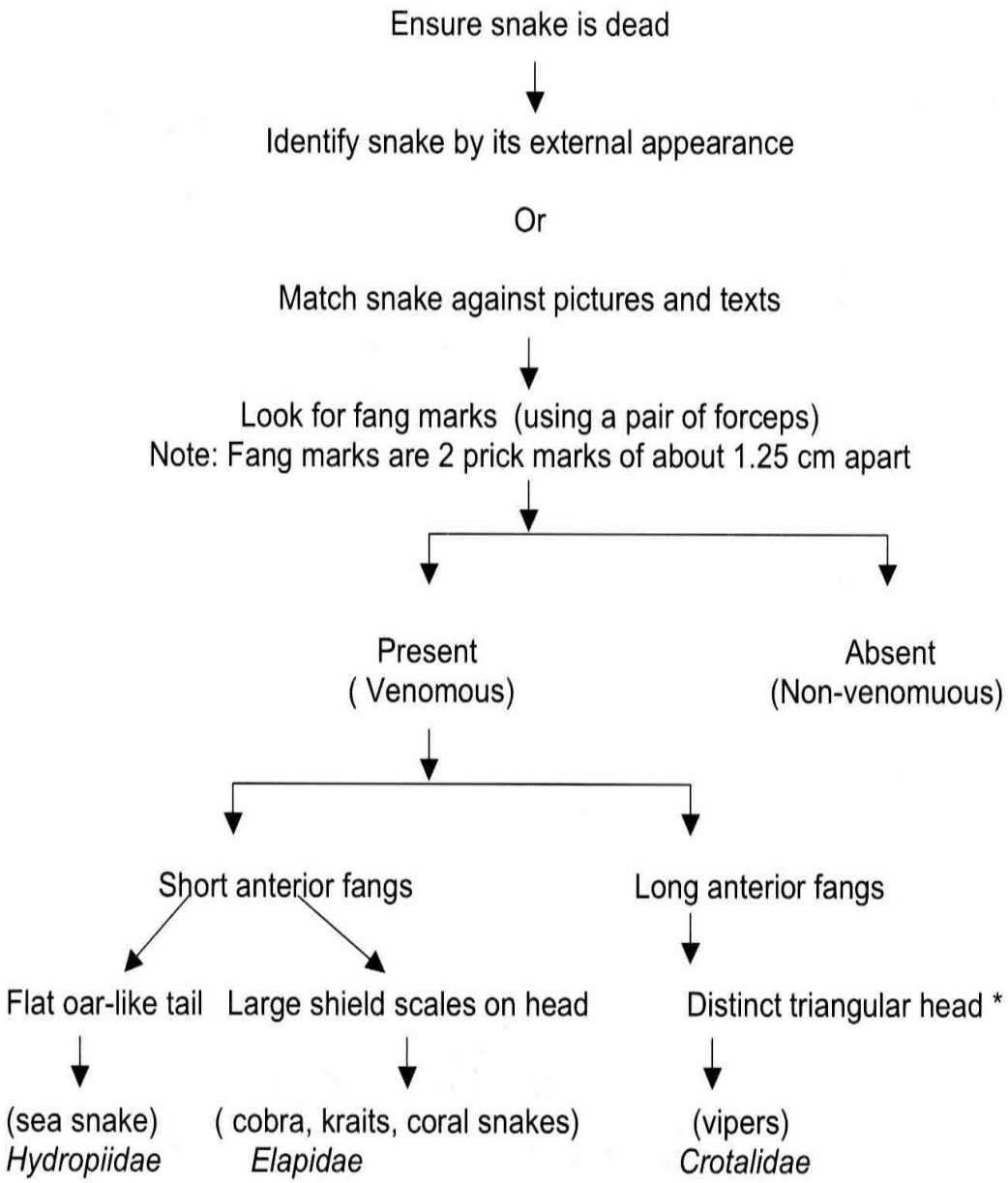
Neurotoxicity, resembling that seen in patients bitten by Elapidae, is seen after bites by *C. d. terrificus, Gloydius blomhoffii, Bitis atropos* and Sri Lankan *D. russelii pulchella*. There may be evidence of generalized rhabdomyolysis. Progression to respiratory or generalized paralysis is unusual.

Late-onset envenoming

The patient should be kept under close observation for at leat 24 hours. Many species, particularly the Krait and the Hump-nosed pitviper (Joseph et al, 2006) are known for the length of time it can take for symptoms to manifest. Often this can take between 6 to 12 hours. Late onset envenoming is a well documented occurrence (Ho et al, 1986) (Warrell et al, 1977) (Reitz, 1989).

This is also particularly pertinent at the start of the rainy season when snakes generally give birth to their young. Juvenile snakes, 8-10 inches long, tend to bite the victim lower down on the foot in the hard tissue area, and thus any signs of envenomation can take much longer to appear.

ALGORITHM: Identification of Snake Bites



* Particularly distinctive features

Laboratory Investigations

The peripheral neutrophil count is raised to 20,000 cells per microlitre or more in severely envenomed patients. Initial haemo-concentration, resulting from extravasation of plasma (*Crotalus* species and Burmese *D. russelii*), is followed by anaemia caused by bleeding or, more rarely, haemolysis. Thrombocytopenia is common following bites by pit vipers (e.g., *C. rhodostoma, Crotalus viridis helleri*) and some Viperidae (e.g., *Bitis arietans* and *D. russelii*), but is unusual after bites by Echis species. A useful test for venom-induced defibrin(ogen)ation is the simple whole blood clotting test. A few millilitres of venous blood is placed in a new, clean, dry, glass test tube, left undisturbed for 20 minutes at ambient temperature, and then tipped to see if it has clotted or not. Incoagulable blood indicates systemic envenoming and may be diagnostic of a particular species (for example Echis species in Africa). The test should be carried out every 30 minutes from admission for three hours and then hourly after that. If incoagulable blood is discovered, the 6 hourly cycle will then be adopted to test for the requirement for repeat doses of ASV

Patients with generalized rhabdomyolysis show a steep rise in serum creatine kinase, myoglobin and potassium. Black or brown urine suggests generalized rhabdomyolysis or intravascular haemolysis. Concentrations of serum enzymes such as creatine phosphokinase and aspartate aminotransferase are moderately raised in patients with severe local envenoming, probably because of

local muscle damage at the site of the bite. Urine should be examined for blood/haemoglobin, myoglobin and protein and for microscopic haematuria and red cell casts.

Treatment

First aid

Patients should be moved to the nearest medical facility as quickly and comfortably as possible, avoiding movement of the bitten limb, which should be immobilized with a splint or sling.

Most traditional first-aid methods are potentially harmful[37] and should not be used. Local incisions and suction may introduce infection, damage tissues and cause persistent bleeding, and are unlikely to remove much venom from the wound. The vacuum extractor method is of unproven benefit in human patients and could damage soft tissues[40][41]. Potassium permanganate and cryotherapy potentiate local necrosis. Electric shock is potentially dangerous and has not proved beneficial. Tourniquets and compression bands can cause gangrene[43][44], fibrinolysis, peripheral nerve palsies and increased local envenoming in the occluded limb[45].

The first aid being currently recommended is based around the mnemonic: **"Do it R.I.G.H.T."** It consists of the following:

R. Reassure the patient. 70% of all snakebites are from non-venomous species. Only 50% of bites by venomous species actually envenomate the patient

I Immobilise in the same way as a fractured limb. Use bandages or cloth to hold the splints, not to block the blood supply or apply pressure. Do not apply any compression in the form of tight ligatures, they don't work and can be dangerous!

G. H. Get to Hospital Immediately. Traditional remedies have NO PROVEN benefit in treating snakebite.

T Tell the doctor of any systemic symptoms such as ptosis that manifest on the way to hospital.

The pressure immobilization method involves firm but not tight bandaging of the entire bitten limb with a crepe bandage 4-5 m long by 10 cm wide starting over the site of the bite and incorporating a splint[38][39]. Pressure immobilization is recommended for bites by snakes with neurotoxic venoms (e.g., *Elapidae*, *Hydrophiidae*) but not when local swelling and necrosis may be a problem (e.g., *Viperidae*)[39].

Patients being transported to hospital should be laid on their side to prevent aspiration of vomit[42]. Persistent vomiting is treated with chlorpromazine by intravenous injection (25 to 50 mg for adults, 1 mg/kg body weight for children). Syncope, shock, angio-oedema and other anaphylactic (autopharmacological)

symptoms are treated with 0.1% adrenaline by subcutaneous injection (0.5 ml for adults, 0.01 ml/kg body weight for children), and an antihistamine such as chlorpheniramine maleate is given by slow intravenous injection (10 mg for adults, 0.2 mg/kg body weight for children). Patients with incoagulable blood develop large haematomas after intramuscular and subcutaneous injections; the intravenous route should be used whenever possible. Respiratory distress and cyanosis are treated by establishing an airway, giving oxygen and, if necessary, assisted ventilation. If the patient is unconscious and no femoral or carotid pulses can be detected, cardiopulmonary resuscitation (CPR) should be started immediately.

Anti Snake Venom (ASV)

Anti snake venom (ASV) in India is polyvalent i.e. it is effective against all the four common species; Russells viper (*Daboia russelii*), Common Cobra (*Naja naja*), Common Krait (*Bungarus caeruleus*) and Saw Scaled viper (*Echis carinatus*). There are no currently available monovalent ASVs primarily because there are no objective means of identifying the snake species, in the absence of the dead snake. It would be impossible for the physician to determine which type of Monovalent ASV to employ in treating the patient.

There are known species such as the Hump-nosed pitviper (*Hypnale hypnale*) where polyvalent ASV is known to be ineffective. In addition, there are regionally

specific species such as Sochurek's Saw Scaled Viper (*Echis carinatus sochureki*) in Rajasthan, where the effectiveness of polyvalent ASV may be questionable.

ASV is produced in both liquid and lyophilised forms[45]. There is no evidence to suggest which form is more effective and many doctors prefer one or the other based purely on personal choice. Liquid ASV requires a reliable cold chain and refrigeration and has a 2 year shelf life. Lyophilised ASV, in powder form, requires only to be kept cool.

ASV Administration Criteria

ASV is a scarce, costly commodity and should only be administered when there are definite signs of envenomation. Unbound, free flowing venom, can only be neutralised when it is in the bloodstream or tissue fluid. In addition, Anti-Snake Venom carries risks of anaphylactic reactions

ONLY if a Patient develops one or more of the following signs/symptoms will ASV be administered [46]

Systemic envenoming

1. Evidence of coagulopathy: Primarily detected by 20WBCT or visible spontaneous systemic bleeding, gums etc.Further laboratory tests for thrombocytopenia, Hb abnormalities, PCV, peripheral smear etc provide confirmation, but 20WBCT is paramount.

   2. Evidence of neurotoxicity: ptosis, external ophthalmoplegia, muscle paralysis, inability to lift the head etc.

The above two methods of establishing systemic envenomation are the primary determinants. They are simple to carry out, involving bedside tests or identification of visible neurological signs and symptoms. In the Indian context and in the vast majority of cases, one of these two categories will be the sole determinant of whether ASV is administered to a patient.

Severe Current Local envenoming

1. Severe current, local swelling involving more than half of the bitten limb (in the absence of a tourniquet). In the case of severe swelling after bites on the digits (toes and especially fingers) after a bite from a known necrotic species.
2. Rapid extension of swelling (for example beyond the wrist or ankle within a few hours of bites on the hands or feet). Swelling a number of hours old is not grounds for giving ASV.

Purely local swelling, even if accompanied by a bite mark from an apparently venomous snake, is not grounds for administering ASV.

Prevention of ASV Reactions – Prophylactic Regimes

Conclusion in respect of prophylactic regimens to prevent anaphylactic reactions, is that there is no evidence from good quality randomized clinical trials to support their routine use.

ASV Administration: Dosage

The recommended dosage level has been based on published research that Russells Viper injects on average 63mg SD 7 mg of venom (Tun Pe, 1986). The

initial dose should be calculated to neutralise the average dose of venom injected. This ensures that the majority of victims should be covered by the initial dose and keeps the cost of ASV to acceptable levels. The range of venom injected is 5mg – 147 mg. This suggests that the total required dose will be between 10 vials to 25 vials as each vial neutralises 6mg of Russells Viper venom. Starting with 10 vials ensures that there is sufficient neutralising power to neutralise the average amount of venom injected and during the next 12 hours to neutralise any remaining free flowing venom[46].

NO ASV TEST DOSE MUST BE ADMINISTERED!

Test doses have been shown to have no predictive value in detecting anaphylactoid or late serum reactions and should not be used (Warrell et al 1999). These reactions are not IgE mediated but Complement activated. They may also pre-sensitise the patient and thereby create greater risk[43][45].

ASV is recommended to be administered in the following initial dose:

Neurotoxic/ Anti Haemostatic	8-10 Vials

N.B. Children receive the same ASV dosage as adults. The ASV is targeted at neutralising the venom. Snakes inject the same amount of venom into adults and children.

ASV can be administered in two ways:

1. Intravenous Injection: reconstituted or liquid ASV is administered by slow intravenous injection. (2ml/ minute). Each vial is 10ml of reconstituted ASV.
2. Infusion: liquid or reconstituted ASV is diluted in 5-10ml/kg body weight of isotonic saline or glucose.

All ASV to be administered over 1 hour at constant speed. The patient should be closely monitored for 2 hours. Local administration of ASV, near the bite site, has been proven to be ineffective, painful and raises the intracompartmental pressure, particularly in the digits. It should not be used[(42)].

ASV Reactions

Anaphylaxis is life-threatening, but despite the reluctance in giving ASV due to reactions (Kalantri et al, 2005), if the correct protocol is followed, it can be effectively treated and dealt with. Anaphylaxis can be rapid onset and can deteriorate into a life-threatening emergency very rapidly. Adrenaline should always be immediately available.

The patient should be monitored closely (Peshin et al, 1997) and at the first sign of any of the following:

Urticaria, itching, fever, shaking chills, nausea, vomiting, diarrhoea, abdominal cramps, tachycardia, hypotension, bronchospasm and angio-oedema

1. ASV will be discontinued
2. 0.5mg of 1:1000 adrenaline will be given IM,

   Children are given 0.01mg/kg body weight of adrenaline IM.

In addition, to provide longer term protection against anaphylactoid reaction, 100mg of hydrocortisone and an H1 antihistamine, such as Phenimarine maleate can be used at 22.5mg IV or Promethazine HCl can be used at 25mg IM, or 10mg chlorphenirmarine maleate if available, will be administered IV.

If after 10 to 15 minutes the patient's condition has not improved or is worsening, a second dose of 0.5 mg of adrenalin 1:1000 IM is given[46]. This can be repeated for a third and final occasion but in the vast majority of reactions, 2 doses of adrenaline will be sufficient. If there is hypotension or hemodynamic instability, IV fluids should be given.

Once the patient has recovered, the ASV can be restarted slowly for 10-15 minutes, keeping the patient under close observation. Then the normal drip rate should be resumed.

The IM route for the administration of adrenaline is the option selected, due to the rapidity of development in anaphylaxis. Studies have shown that adrenaline reaches necessary blood plasma levels in 8 minutes in the IM route, but up to 34 minutes in the subcutaneous route (American Association, 2003) (Simons, 1998). The early use of adrenaline has been selected as a result of study evidence suggesting better patient outcome if adrenaline is used early (Sampson et al, 1992).

In extremely rare, severe life threatening situations, 0.5mg of 1:10,000 adrenaline can be given IV. This carries a risk of cardiac arrhythmias however,

and should only be used if IM adrenaline has been tried and the administration of IV adrenaline is in the presence of ventilatory equipment and ICU trained staff.

It is widely believed that anaphylactoid reactions are under reported (McLean-Tooke et al, 2003)

Late Serum sickness reactions can be easily treated with an oral steroid such as prednisolone, adults 5mg 6 hourly, paediatric dose 0.7mg/kg/day. Oral H1 Antihistamines provide additional symptomatic relief.

Neurotoxic Envenomation

Neostigmine is an anticholinesterase that prolongs the life of acetylcholine and can therefore reverse respiratory failure and neurotoxic symptoms. It is particularly effective for post synaptic neurotoxins such as those of the Cobra (Watt et al, 1986). There is some doubt over its usefulness against the pre-synaptic neurotoxin such as those of the Krait and the Russells Viper (Warrell et al, 1983) (Theakston et al, 1990). However it is worth trying in these cases.

In the case of neurotoxic envenomation the 'Neostigmine Test' will be administered, 1.5-2.0 mg of neostigmine IM, together with 0.6mg of atropine IV. The patient should be closely observed for 1 hour to determine if the neostigmine is effective.

The following measures are useful objective methods to assess this:

a) Single breath count

b) Mm of Iris uncovered (Amount covered by the descending eyelid)

c) Inter incisor distance (Measured distance between the upper and lower incisors)

d) Length of time upward gaze can be maintained

e) FEV 1 or FVC (If available)

The average blood plasma time for neostigmine is 20 minutes, so by T+30 minutes any improvement should be visible by an improvement in the measure[46].

If the victim responds to the neostigmine test then continue with 0.5mg of neostigmine IM half hourly plus 0.6mg of atropine IV over an 8 hour period by continuous infusion. If there is no improvement in symptoms after one hour, the neostigmine should be stopped.

Repeat Doses: Anti Haemostatic

In the case of anti haemostatic envenomation, the ASV strategy will be based around a six hour time period. When the initial blood test reveals a coagulation abnormality, the initial ASV amount will be given over 1 hour.

No additional ASV will be given until the next Clotting Test is carried out. This is due to the inability of the liver to replace clotting factors in under 6 hrs.

After 6 hours a further coagulation test should be performed and a further dose should be administered in the event of continued coagulation disturbance. This dose should also be given over 1 hour. CT tests and repeat doses of ASV should continue on a 6 hourly pattern until coagulation is restored, unless a species is identified as one against which Polyvalent ASV is not effective.

The repeat dose should be 5-10 vials of ASV i.e. half to one full dose of the original amount. The most logical approach is to administer the same dose again, as was administered initially.

Repeat Doses: Neurotoxic

If the initial dose has been unsuccessful in reducing the symptoms or if the symptoms have worsened or if the patient has gone into respiratory failure then a further dose should be administered, after 1-2 hours. This dose should be the same as the initial dose, i.e. if 10 vials were given initially then 10 vials should be repeated for a second dose and then ASV is discontinued. 20 vials is the maximum dose of ASV that should be given to a neurotoxically enven0omed patient.

Once the patient is in respiratory failure, has received 20 vials of ASV and is supported on a ventilator, ASV therapy should be stopped. Therefore further ASV serves no useful purpose.

Surgical Intervention

Whilst there is undoubtedly a place for a surgical debridement of necrotic tissue, the use of fasciotomy is highly questionable. The appearance of (Joseph, 2003):

- Pain on passive stretching
- Pain out of proportion

- Pulselessness
- Pallor
- Parasthesia
- Paralysis

with significant swelling in the limb, can lead to the conclusion that the intracompartmental pressure is above 40 mm of mercury and thus requires a fasciotomy. Fasciotomy is required if the intracompartmental pressure is sufficiently high to cause blood vessels to collapse and lead to ischemia. Fasciotomy does not remove or reduce any envenomation.

Materials and Methods

Setting : PCTRC, Government General Hospital & Madras Medical College, Chennai

Design of study : Descriptive study

Duration of study : January 2009 to June 2009

Ethical Clearance : Obtained from institutional authorities

Informed consent : Obtained

Materials :

Consecutive patients admitted to PCTRC with one or other neurological manifestations following snake envenomation over a period of six months commencing from January 2009 formed the materials for the study.

Exclusion :

Non venomous snakebites and venomous snakebites with non neurological manifestations were not included in the study

Pregnant women and persons with any co-existing illness were excluded.

Methods :

All patients admitted with neurological manifestations due to snakebite were studied . The study was carried out over a six-month-period beginning January 2009 up to June 2009. The offending snakes were identified either by direct examination (when the snake was killed and brought to the hospital) or on the basis of eye-witness account. This evidence was further be verified by showing photographs of snakes to the eye witness.

Detailed history was obtained and patients were subjected to neurological

examination soon after admission. They were regularly assessed hourly for the first six hours, 12 hourly for next 72 hours, and then daily until complete recovery. Information regarding progression, onset of recovery and results of biochemical investigations were entered in a pre-designed proforma. All patients underwent intravenous neostigmine challenge test, whose results were also noted. All patients were treated with lyophilised polyvalent enzyme refined equine immunoglobulins (antivenom serum; Haffkine Institute, Mumbai, India)

Electrophysiologic studies were done on all the patients and the correlation between clinical and electrophysiologic progression analysed. The following studies were carried out

1. Motor nerve conduction studies in median and ulnar nerve
    i) measurement of compound muscle action potential (CMAP) amplitude
    ii) measurement of F wave latencies
    iii) calculation of motor conduction velocities
2. Sensory nerve conduction studies in median and ulnar nerves
    i) measurement of sensory nerve action potential (SNAP)
    ii) calculation of sensory nerve conduction velocities
3. Repetitive nerve stimulation tests
    i) Recording the change in CMAP amplitude recorded in abductor digiti minimi after a train of stimuli at 3 Hz to the ulnar nerve at the wrist

Data Collection :

a) Demographic data : Age, sex, domicile, socio economic status

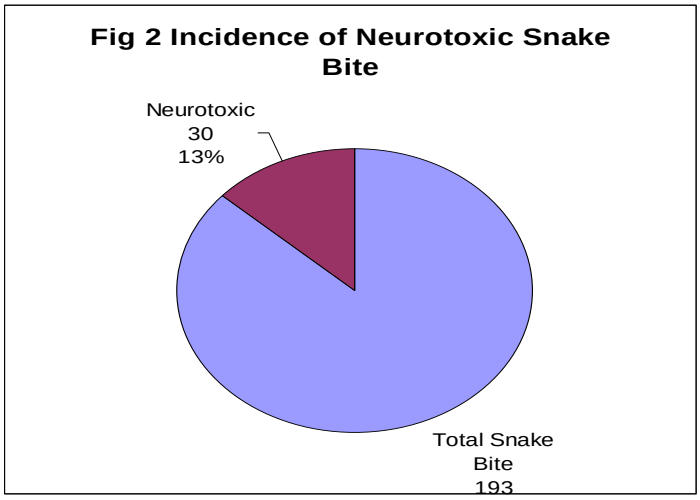
b) Clinical data : Offending snake (wherever feasible), Clinical Features, Associated toxicities, Bite to Neurotoxicity time, Bite to ASV time, electro-physiologic changes, disease course and outcome

c) Statistics : Simple descriptive statistics

Results

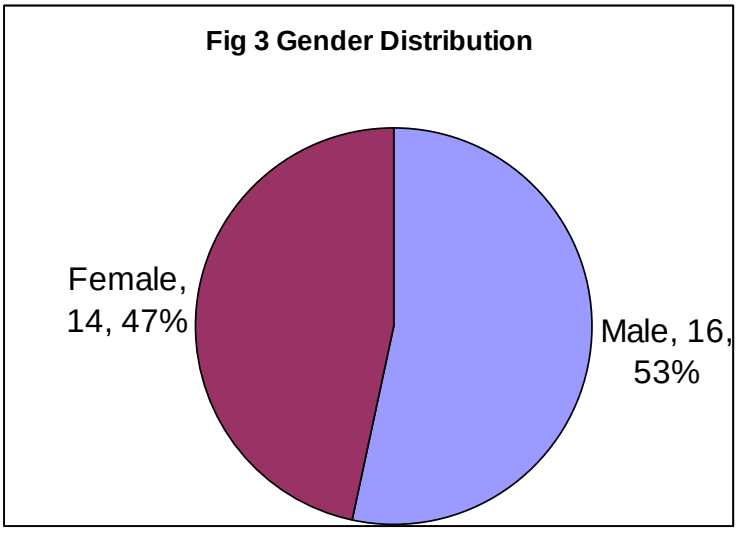
Magnitude of the problem :

During the study period a total of 193 patients were admitted to our centre with Snake bite. Of these 193 patients, 30 patients had neurotoxic features either alone or associated with other features such as hemotoxicity or local cellulites. .



GENDER DISTRIBUTION

Out of the 30 patients, 21 patients were males making up 70% of the cases. There were 9 females making up the remainder 30% of the cases.



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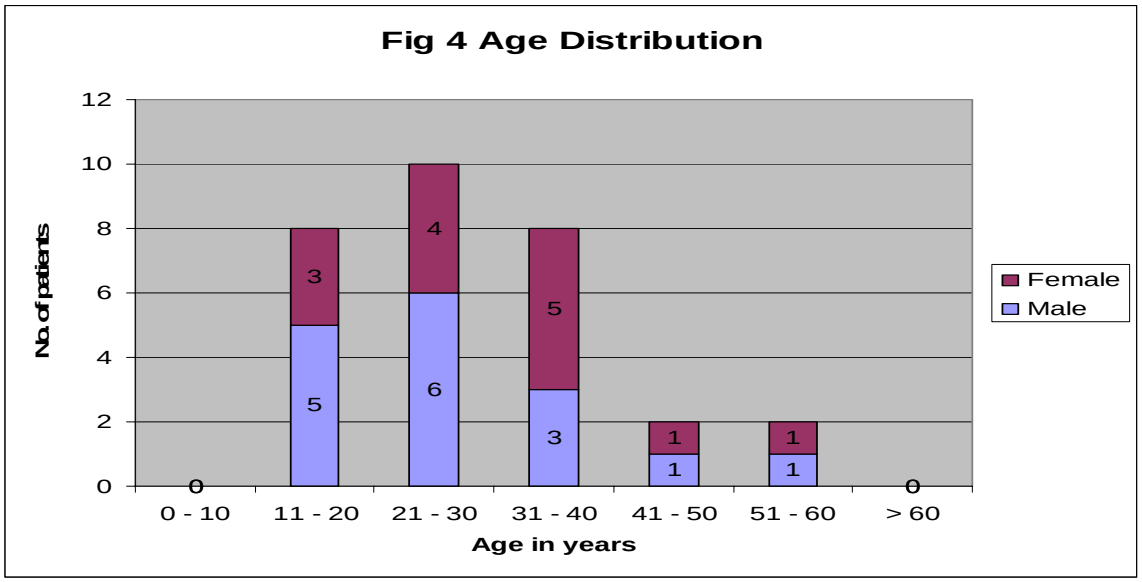
AGE DISTRIBUTION

Patients were distributed between 17 & 60 years of age with a mean age – 30.35 and SD – 10.8.

S no.	Age Group in Years	No. of Patients
1	< 10	0
2	11 – 20	8
3	21 – 30	10
4	31 - 40	8
5	41 – 50	2
6	51 – 60	2
7	> 60	0

Table 2 Age Distribution

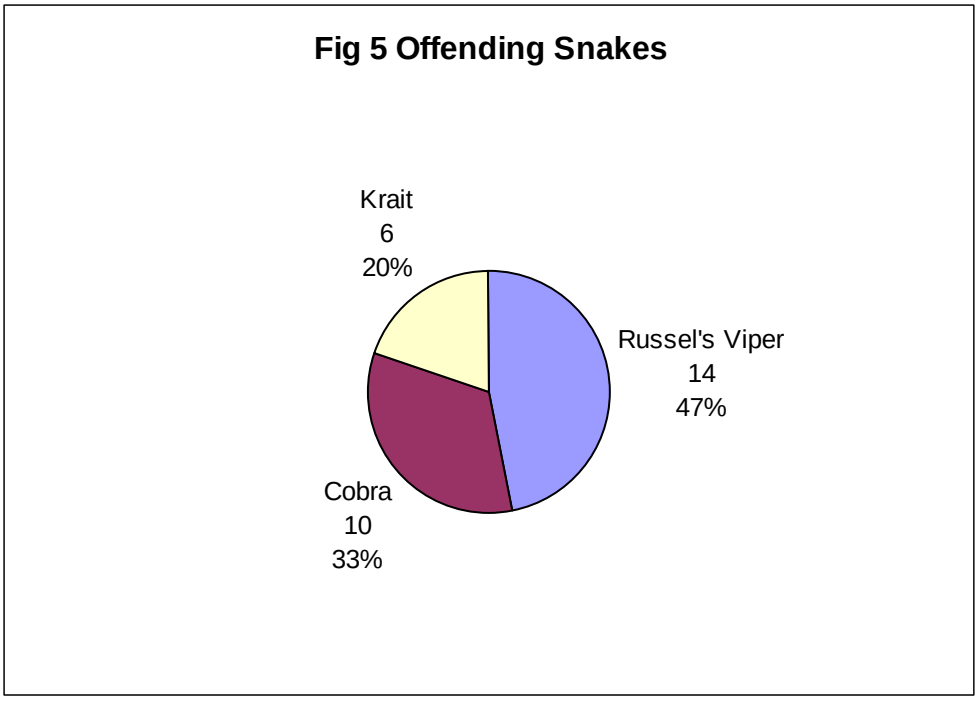


OFFENDING SNAKES

The offending snake was identified by direct examination in just 5 cases and the remaining by eye witness account. The offending snakes were identified as due to Russels viper in 14 (46.7%), Cobra in10 (33.3%) and Krait in 6 (20%)

S. No.	Offending Snake	Number of Patients	Percentage
1	Russels viper	14	47%
2	Cobra	10	33%
3	Krait	6	20%

Table 3 Offending Snakes



PRESENTING FEATURES

Ptosis was the most common presenting feature followed by ophthalmoplegia, limb, neck & respiratory muscles in that order.

Signs of local envenomation were demonstrable in 80% of patients. Among them 12 had associated hemotoxicity.

S. No.	Presenting Feature	Relative Frequency
1	Ptosis	90%
2	Ophthalmoplegia	80%
3	Cellulitis	80%
4	Limb Weakness	46.7%
5	Hemotoxicity	40%
6	Neck&Resp.Muscle	33.3%
7	Complete paralysis	20%
8	Renal Failure	10%

Table 4 Clinical Features

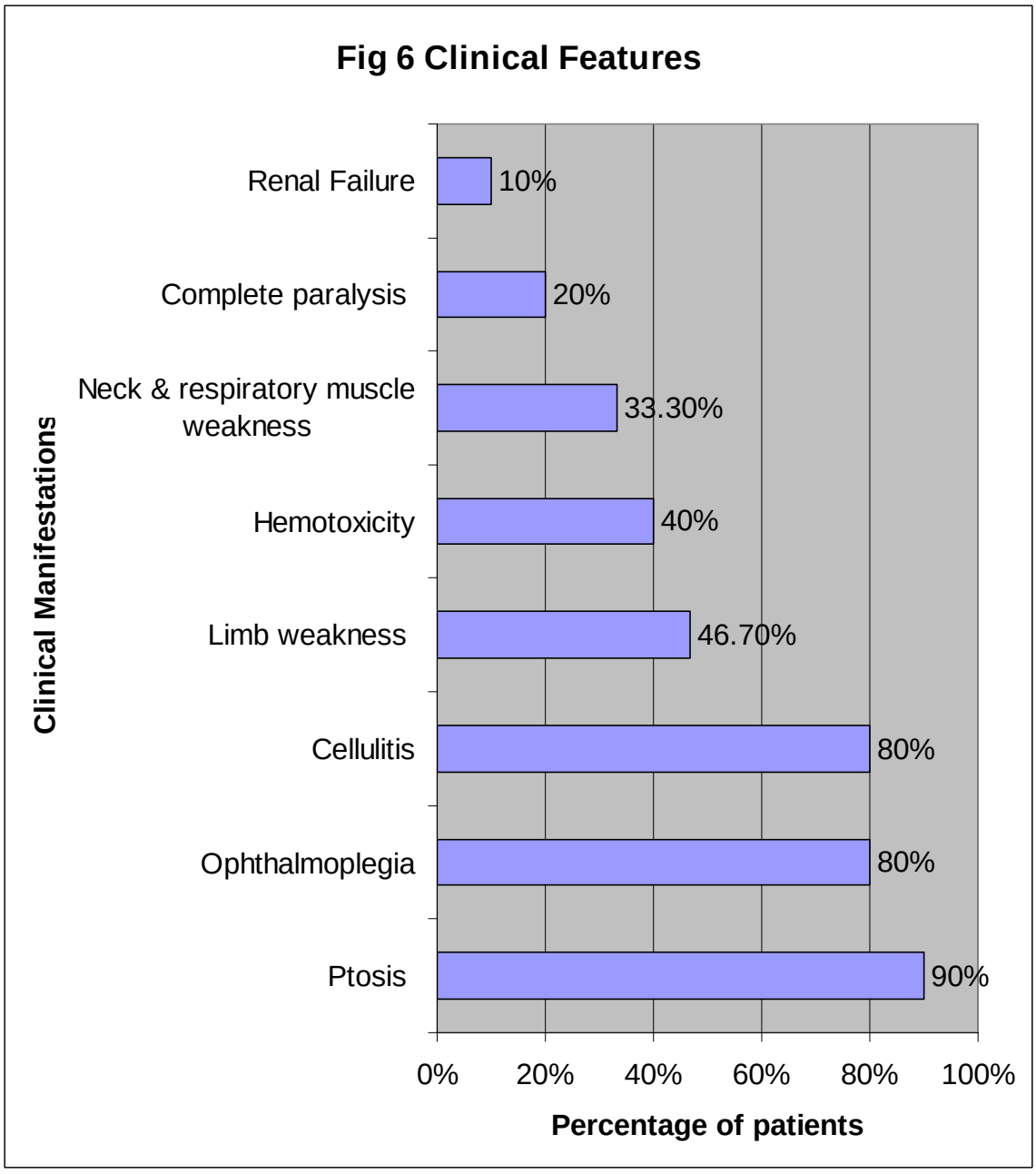
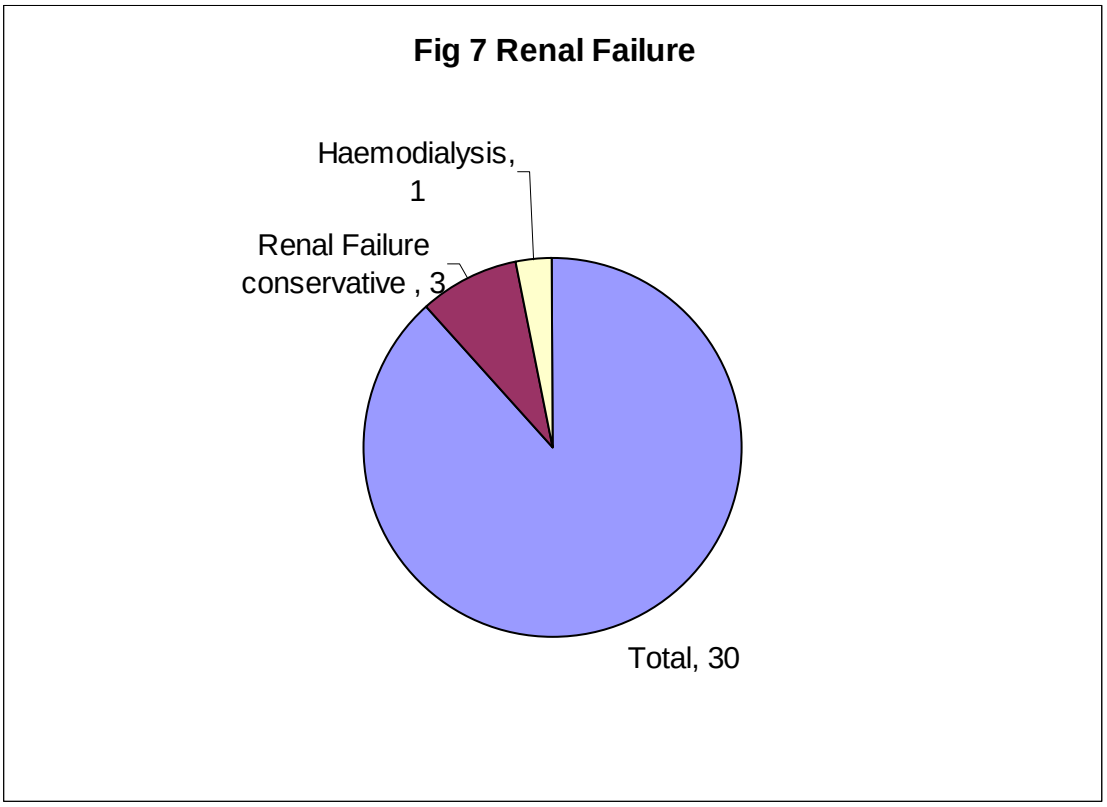


Fig 6 Clinical Features
Clinical Manifestations
Renal Failure
10%
Complete paralysis
20%
Neck & respiratory muscle weakness
33.30%
Hemotoxicity
40%
Limb weakness
46.70%
Cellulitis
80%
Ophthalmoplegia
80%
Ptosis
90%
0%
20%
40%
60%
80%
100%
Percentage of patients

Of these patients with hemotoxicity, 4 patients developed renal failure; 3 of the 4 patients with renal failure recovered with conservative management and one patient needed hemodialysis.

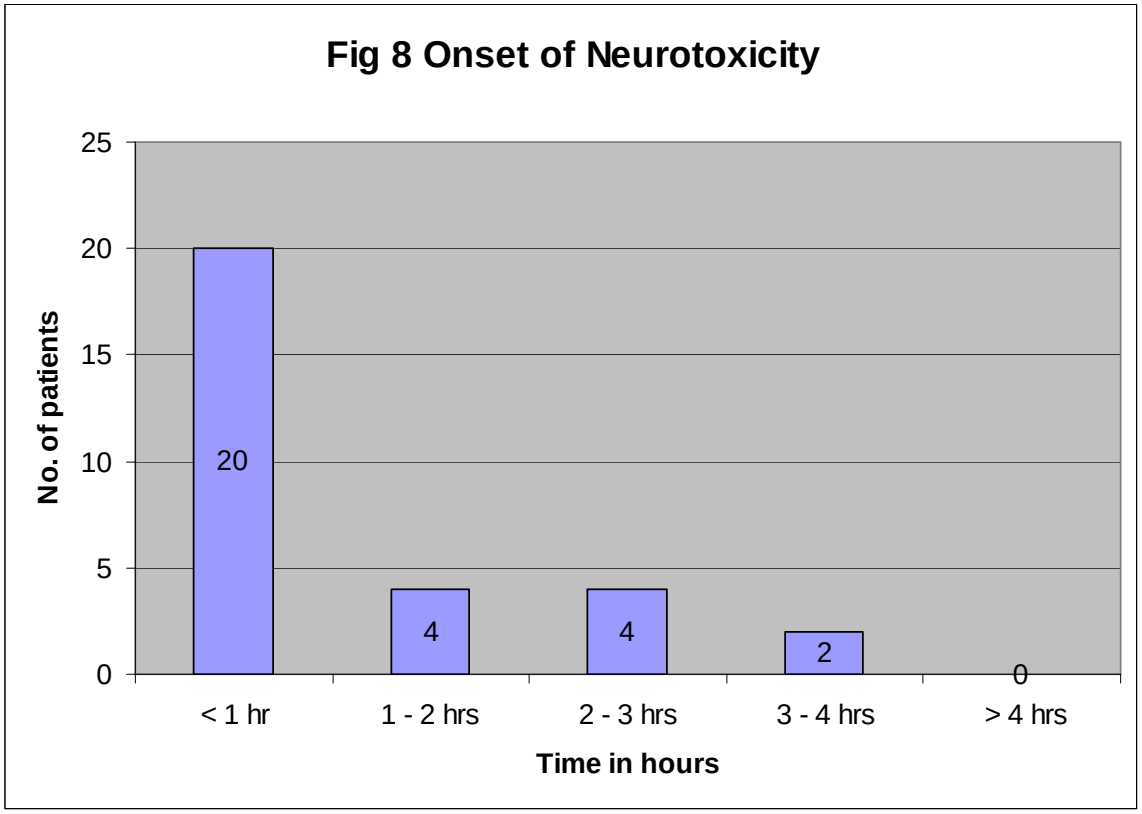


BITE TO NEUROTOXICITY

Neurological features developed within 30 minutes to 4 hours from the time of bite. 20 patients (67%) developed neurological features with in the first hour of bite. 4 patients (13%) developed features between 1 and 2 hours, another 4 patients (13%) between 2 and 3 hours and 2 patients (7%) between 3 and 4 hours.

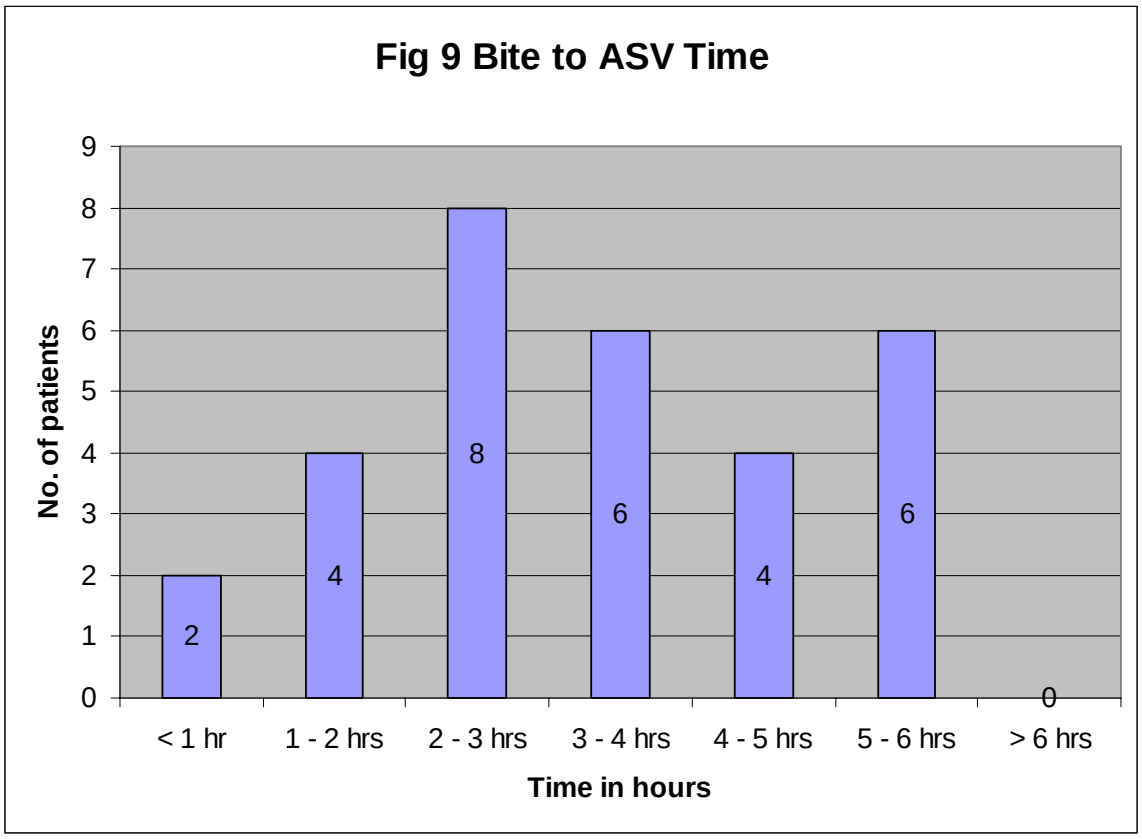
S. No.	Time Interval	No. of Patients
1	<1 hr	20
2	1 – 2 hrs	4
3	2 – 3 hrs	4
4	3 – 4 hrs	2
5	> 4 hrs	0

Table 5 Bite to Neurotoxicity



BITE TO ASV TIME

All the patients who showed features of toxicity needing Anti snake Venom received the first dose of Anti snake venom within 6 hours of bite, either at the nearest health facility or at our centre, the mean being 3.7 hours (SD – 1.74).

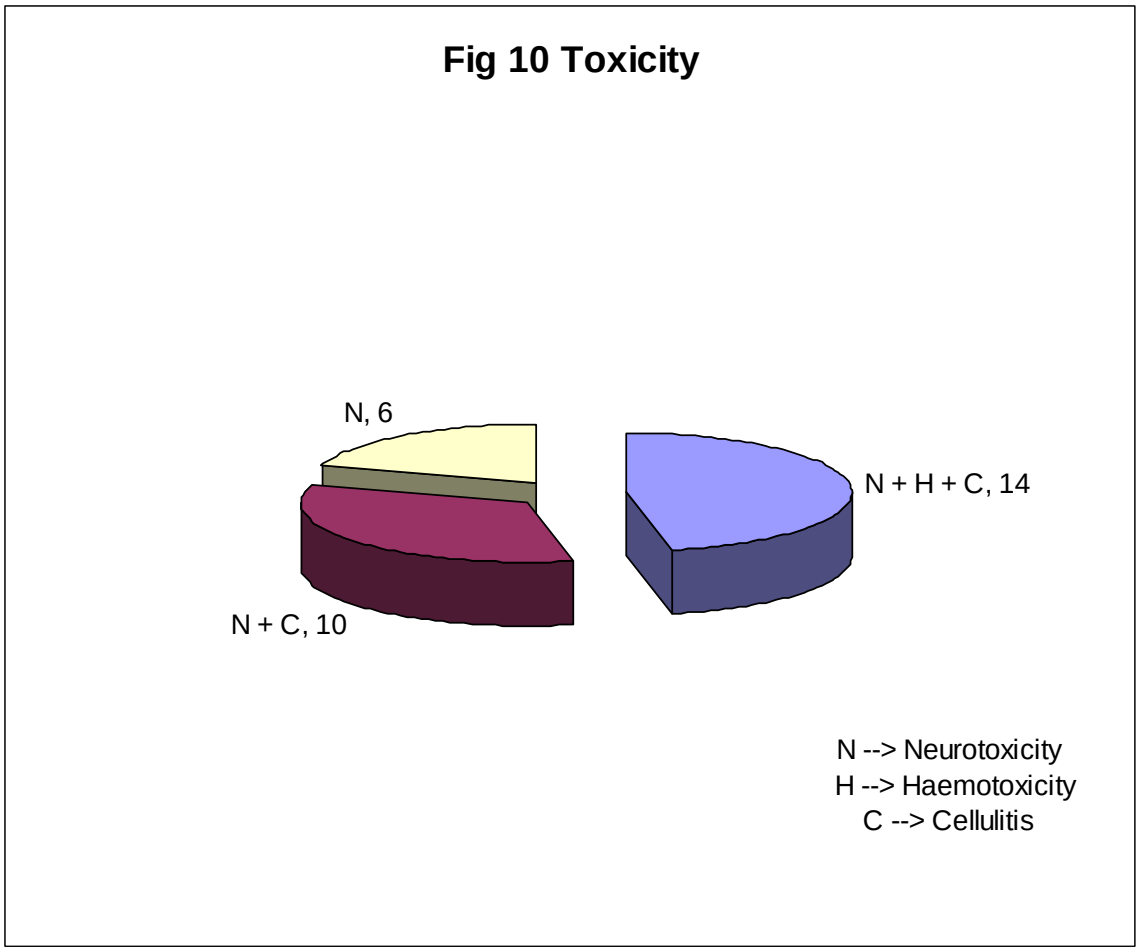


MECHANICAL VENTILATION

Of the 10 patients who had respiratory muscle weakness, only five required mechanical ventilation support on arrival at PCTRC.

PROFILE IN PATIENTS WITH CONCOMITANT HEMOTOXICITY

Among the patients who showed features of Hemotoxicity and Local Cellulitis in addition to Neurotoxic features, it was observed that the neurotoxicity was less severe than in those who had associated toxicities. Ptosis (75%) and ophthalmoplegia (66.6%) were found to be the most common manifestation. None of them developed respiratory manifestations.



ELECTROPHYSIOLOGIC CHANGES

Nerve conduction studies were performed on all 30 patients who showed neurotoxic features. Of these 30 patients with neurotoxicity, Nerve conduction studies were abnormal in 21 patients. Neurotoxicity was completely reversed in the remaining 9 patients by the time NCS was performed.

The following parameters were studies in the patients

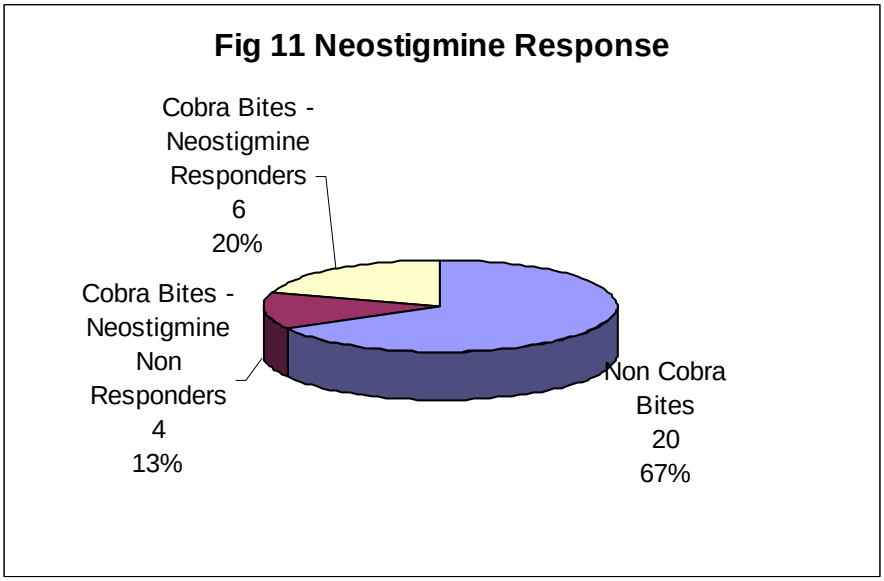
1. Motor nerve conduction studies in median and ulnar nerve
    i) measurement of compound muscle action potential (CMAP) amplitude
    ii) measurement of F wave latencies
    iii) calculation of motor conduction velocities
2. Sensory nerve conduction studies in median and ulnar nerves
    i) measurement of sensory nerve action potential (SNAP)
    ii) calculation of sensory nerve conduction velocities
3. Repetitive nerve stimulation tests
    i) Recording the change in CMAP amplitude recorded in abductor digiti minimi after a train of stimuli at 5 Hz to the ulnar nerve at the wrist

It was observed that CMAP was decreased in 15 patients (50%) out of the 21 patients with abnormal NCS. 6 patients (28.6%) out of the 21 showed decremental response on RNS and another 6 (28.6%) showed incremental response. 9 patients (42.9%) out of the 21 did not show changes on RNS. No changes were observed in the F wave latencies, motor & sensory nerve conduction velocities or sensory

nerve action potential. There was no evidence of delayed sensory or motor neuropathy in any of them.

Offending Snake	Normal NCS	Decreased CMAP	Decremental RNS	Incremental RNS
Russel's viper	6	7	0	2
Cobra	3	4	6	1
Krait	0	4	0	3
Total	9	15	6	6

Table 6 Nerve Conduction Changes



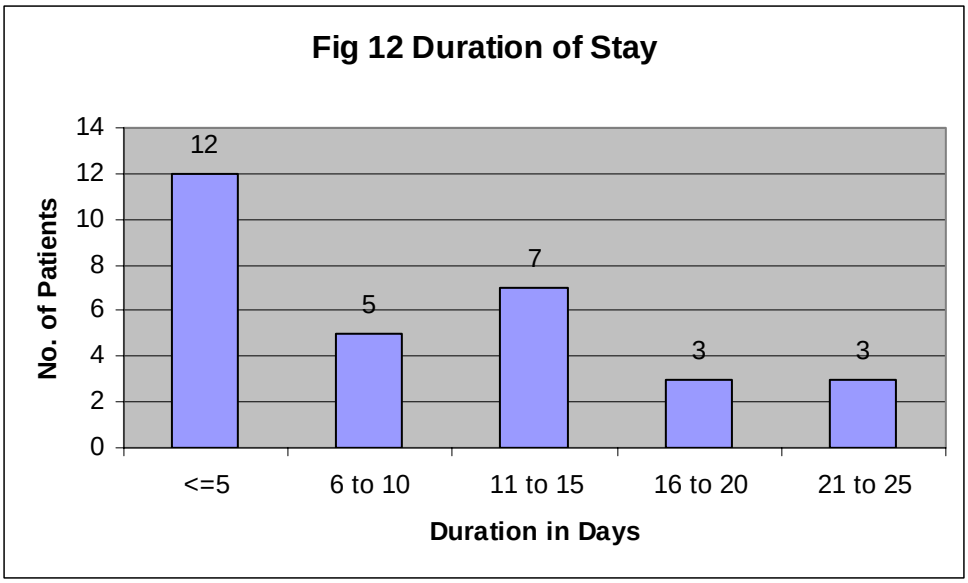
The 6 paients who showed a decremental response on RNS were victims of cobra bite. However, the 4 other victims of cobra bite failed to show such a response. The same six patients bitten by Cobra, who showed decremental response on NCS also responded to neostigmine. Thus they correlated with the pattern of post synaptic blockade on nerve conduction studies.

DURATION OF STAY

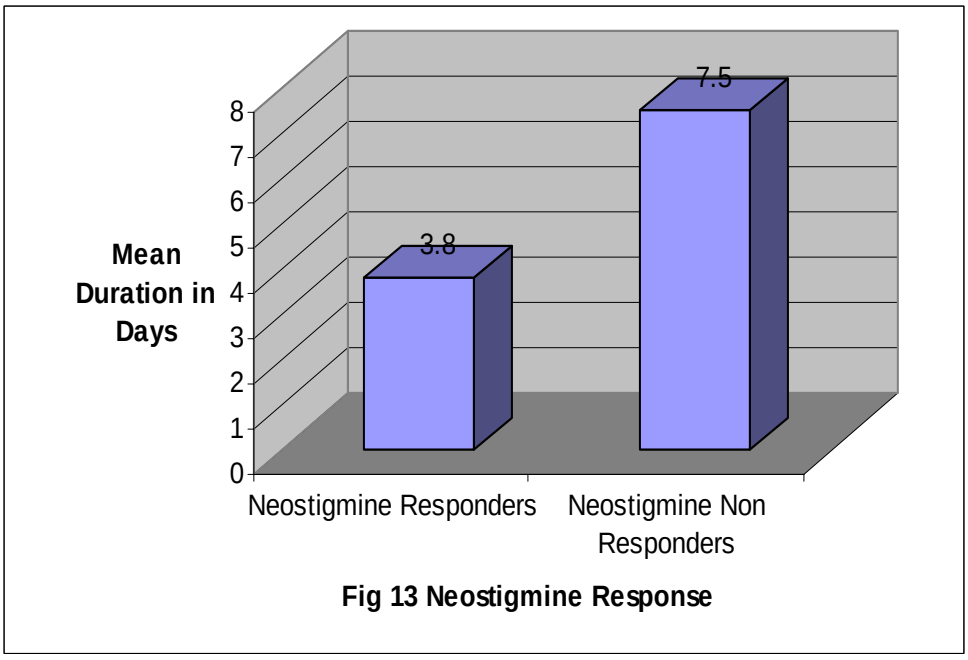
The over all length of stay in hospital varied from 3 to 22 days with a mean of 7.5 days (SD – 2.2).

S. No.	Duration in Days	No. of Patients
1	<= 5 days	12
2	6 – 10 days	6
3	11 – 15 days	7
4	16 – 20 days	3
5	21 – 25 days	3

Table 7 Duration of stay



The length of stay (mean - 3.8 days, SD – 1.3) was significantly low among neostigmine responsive patients (n=6, 20%) than among non responders (P=0.01).



DEATHS

There were no deaths during the study period in our study. All the patients who went in for respiratory distress requiring mechanical ventilation and hemodialysis were successfully treated and recovered without any residual sequelae.

Discussion

At least 421,000 envenomings and 20,000 deaths occur worldwide from snakebite annually. These figures may be as high as 1,841,000 envenomings and 94,000 deaths. On the basis of the estimation that the total number of snakebites is two to three times the number of envenomings**,** it is estimated that 1,200,000–5,500,000 snakebites may occur globally. The vast majority of the estimated burden of snakebite is in South and Southeast Asia, sub-Saharan Africa, and Central and South America, In our study, a total of 193 patients were admitted with Snake bite. Of these 193 patients, 30 patients had neurotoxic features either alone or associated with other features such as hemotoxicity or local cellulites. .
Out of the 30 patients, 21 patients were males making up 70% of the cases. A similar finding was observed by Dissenayake et al where males made up 80.4% of the cases. There were 9 females making up the remainder 30% of the cases in our study.

Our patients were distributed between 17 & 60 years of age with a mean age – 30.35 and SD – 10.8. In another study done by Seneviratne U et al, the Mean age was found to be 31.9 years with S.D.- 12.1

In our study, the offending snake was identified by direct examination in just 5 cases and the remaining by eye witness account. The offending snakes were identified as due to Russels viper in 14 (46.7%), Cobra in10 (33.3%) and Krait in 6 (20%). As in most cases, the snake was identified on the basis of eye-witness accounts we have not made an attempt to strictly correlate all clinical findings

with snake species as the above method of identification may not be absolutely foolproof, even though inhabitants of the area are very familiar with snakes.

In our series, we noticed that Ptosis was the most common presenting feature (90% of patients had ptosis) followed by ophthalmoplegia, limb, neck & respiratory muscles in that order. In the study by Seneviratne U et al also, the most common symptom was drooping of eyelids (85.7%) followed by double vision and dysphagia

Neurological features developed within 30 minutes to 4 hours from the time of bite in all our patients. 20 patients (67%) developed neurological features with in the first hour of bite. 4 patients (13%) developed features between 1 and 2 hours, another 4 patients (13%) between 2 and 3 hours and 2 patients (7%) between 3 and 4 hours. In the study done by The Department of Neurology, Ratnapura General Hospital, Ratnapura and Base Hospital, Polonnaruwa, Sri Lanka, Nine patients (16.1%) developed neurological symptoms within 30 minutes of the bite whereas 26 (46.4%) reported symptoms from 30 minutes to two hours after the bite. The onset of symptoms was from two to four hours after the bite in 13 (23.2%). In a minority (8 cases; 14.2%) symptoms occurred after four to six hours.

All the patients in our study who showed features of toxicity needing Anti snake Venom received the first dose of Anti snake venom within 6 hours of bite, either at the nearest health facility or at our centre, the mean being 3.7 hours (SD – 1.74).

Of the 10 patients who had respiratory muscle weakness, only five required mechanical ventilation support on arrival at PCTRC. In the study by Dissenayake et al, 10 patients developed respiratory muscle weakness necessitating mechanical ventilation. The majority of them were bitten by the krait. Respiratory muscle weakness is a potentially fatal manifestation of snake bite. Krait bite is especially notorious for rapid development of respiratory failure. However, in our series all patients with respiratory failure were successfully managed with the help of mechanical ventilation This emphasises the importance of anticipation of this complication and timely intervention

Snake venom is not a single toxin but a complex mixture of several components, including enzymes, polypeptide toxins, non-toxic proteins, carbohydrates, metals, lipids, free amino acids, nucleotides and bionic amines[17]. Neurotoxins of snake venom seem to affect various sites of neuromuscular system. Snake venom neurotoxins that bind to acetylcholine receptor sites on the motor endplate produce effects similar to those of curare and myasthenia gravis[10]. Another group of neurotoxins with phospholipase A2 bind presynaptically, thereby depressing transmitter release, and are completely resistant to anticholinesterases[10][11][12].

The venom from neurotoxic snake bites can be broadly classified in to a-, b- or k- bungarotoxins. While a- and k- bungarotoxins bind to the post synaptic nicotinic AchR at the neuromuscular junction and neuronal tissue respectively, b-bungaroroxin binds to the the presynaptic terminal blocking the release of acetyl

choline. These toxins show almost irreversible binding to the receptors, competitively inhibiting acetylcholine binding and, consequently, inhibiting the acetylcholine-induced electrical response. Snake venom makes use of AChE to breakdown any neurotransmitter that might compete with a- or k-bungarotoxin for binding to AchRs[10][11][12]. Myotoxic effects causing rhabdomyolysis has been reported. Clinical and electromyographic evidence of myokymia have been demonstrated in victims of timber rattlesnake bite (Crotalus horridus horridus) suggesting increased peripheral nerve terminal excitability.

There is substantial clinical and electrophysiological evidence of defective neuromuscular transmission, both pre-synaptic as well as post-synaptic, in neurotoxic envenomation.

The findings from the nerve conduction studies in our series demonstrate both pre and post synaptic pattern of neuromuscular blockade.

In our series, it was observed that CMAP was decreased in 15 patients (50%) out of the 21 patients with abnormal NCS. A similar finding was seen in the study by Singh et al based on the electrophysiological assessment in patients with Bungarus caeruleus bite[8] .

Six patients (28.6%) out of the 21 showed decremental response on RNS and another 6 (28.6%) showed incremental response. 9 patients (42.9%) out of the 21 did not show changes on RNS

Decremental responses on RNS have been shown in a number of studies in envenomation by different snakes, the Papuan taipan snake (Oxyuranus scutellatus

canni)[9] , Philippine cobra (Naja naja philippinensis)[3], A study from Sri Lanka also showed decremental response to RNS at 20 Hz and 50 Hz.[4] .

In the current study 6 patients showed improvement with neurotoxicity following neostigmine administration and also a significant decrease in the mean length of stay in the hospital. A similar finding has been observed in a previous studies on neuroparalytic envenomation[3][13] while some studies have not proved any benefit[14]

No changes were observed in the F wave latencies, motor & sensory nerve conduction velocities or sensory nerve action potential in our series.

The 6 paients who showed a decremental response on RNS were victims of cobra bite. However, the 4 other victims of cobra bite failed to show such a response. The same six patients bitten by Cobra, who showed decremental response on NCS also responded to neostigmine.

There was no evidence of delayed sensory or motor neuropathy in any of them. A study by Seneviratne et al described a patient with delayed sensory neuropathy in a patient following krait bite[16]. There have also been reports of Guillain Barre syndrome[6] and delayed neuropathy[7][16] in patients with snake bite. However, the current study did not show any findings similar to the ones described above. It could be postulated that either direct neurotoxicity or a reaction to antivenom may have been responsible for these manifestations found in the above studies. Adverse reactions to antivenom appear in two forms; early and late. Early reactions tend to occur within 10 to 180 minutes after treatment and

range from urticaria to anaphylactic shock. Late reactions are immune complex diseases and present in the form of serum sickness syndrome usually 5 to 24 days after antivenom administration. Both central and peripheral nervous system manifestations are seen in association with serum sickness. In view of delayed onset, some form of immune-mediated neuropathy is perhaps more likely. Autoantibody assays such as anti ganglioside antibodies, immune complex studies, serial electrophysiology and nerve biopsy would be useful to elucidate the mechanism of delayed neuropathy following neurotoxic envenomation.

In the current study, 6 patients showed incremental response on RNS. Although the findings may be correlated with the presynaptic pattern of neuromuscular blockade, none of the previous studies mentioned above demonstrated a similar finding.

The over all length of stay in hospital varied from 3 to 22 days with a mean of 7.5 days (SD – 2.2). The length of stay (mean - 3.8 days, SD – 1.3) was significantly low among neostigmine responsive patients (n=6, 20%) than among non responders (P=0.01). A similar finding has been observed in a previous studies on neuroparalytic envenomation (Watt G 1986, Pandey AK 1979) while some studies have not proved any benefit (Sethi PK 1981)

There were no deaths during the study period in our study. All the patients who needed mechanical ventilatory support and hemodialysis recovered completely with out any residual sequelae. This emphasises the importance of anticipation of this complication and timely intervention.

Conclusion

- Early recognition and timely administration of Anti snake venom reduces the neurological complications.
- There was no evidence of delayed motor or sensory neuropathy in any of the patients. Neostigmine responsive patients had shorter duration of stay in the hospital.
- The development of neurological complications is influenced by the nature and quantity of envenomation, susceptibility of the individual, post-synaptic status, and delay in administration of anti-venin.
- In view of the responses on NCS obtained in the current study, further laboratory studies are necessary to isolate and characterise the neurotoxic components of snake venom,
- An understanding of the molecular basis will facilitate our understanding of snake venom neurotoxin and its therapeutic implications, both in treatment of snake bites and in the development of a novel neuromuscular blocking agent.
- Further, community based intervention programmes will reduce the occurrence of snake bite, morbidity and mortality, and length of stay in hospital .

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Annexures

LIST OF ABBREVIATIONS

AchR	Acetylcholine receptor
a- bungaroroxin	alpha bungarotoxin
b- bungaroroxin	beta bungarotoxin
k- bungarotoxin	kappa bungarotoxin
WBCT	Whole blood clottong time
NGF	nerve growth factor
NCS	Nerve conduction studies
CMAP	Compound muscle action potential
SNAP	Sensory nerve action potential
RNS	Repetitive nerve stimulation
MNCV	Motor nerve conduction velocity
SCNV	Sensory nerve conduction velocity
AchE	Acetylcholine Esterase
EMG	Electromyogram
HD	Hemodialysis
ASV	Anti snake venom
M receptors	Muscarinic receptors
N receptors	Nicotinic receptors

LIST OF TABLES

LIST OF FIGURES

PROFORMA

Name Age Sex

Address Occupation

Offending Snake

- Russels viper
- Cobra
- Krait
- Others
- Unidentified

Presenting Complaints

- Ptosis
- Ophthalmoplegia
- Cellulitis
- Limb muscle weakness
- Bleeding Tendencies
- Neck & Respiratory muscle weakness
- Complete Paralysis
- Renal Failure

Duration between bite and onset of Clinical Features

Duration between snake bite and first dose of ASV

Nerve Conduction Changes

- CMAP
- MNCV
- F wave latencies
- SNAP
- SNCV
- RNS

Need for Mechanical Ventilation

Need for Hemodialysis

Duration of stay in the hospital

Outcome

INSTITUTIONAL ETHICAL COMMITTEE
MADRAS MEDICAL COLLEGE,CHENNAI-600 003.

Telephone : 25363970

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:044 25363970

L.Dis.No. 14597/MES/EthicsDean/MMC/2009 Dated .09.2009

Till of the work : "Electrophysiologic changes in patients admitted with neurotoxic snake bite"

Principal Investigator : Dr. K. prashanth. p.g. (M.D - Internal medicine)

Department : Madras medical college, ch-3

The request for an approval from the Institutional Ethical Committee(IEC) was considered on the IEC meeting held on 23rd September 2009 at 2.00P.M. in Madras Medical College, Deans, Chamber, Chennai-3./ pharmacology seminar hall-madras medical college-ch-3.

The members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The principal investigator and their term are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate form the area of the work for which I applied for ethical clearnance.
5. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
6. You should abide to the rules and regulations of the institution(s).
7. You should complete the work within the specific period and if any extension of time is required, you should apply for permission again and do the work.
8. You should submit the summary of the work to the ethical committee on completion of the work.
9. You should not claim funds from the Institution while doing the work or on completion.
10. You should understand that the members of IEC have the right to monitor the work with prior intimation.

SECRETARY
IEC, MMC,CHENNAI

CHAIRMAN
IEC MMC CHENNAI

DEAN 21/10/09
MADRAS MEDICAL COLLEGE
CHENNAI

name	age	sex	address	species	hemo	neuro	cellulitis	renal	ptosis	ophthalm	neck	resp dis	complete
Kannaiyan	60	m	sub urban	cobra	-	+	+	-	+	+	+	+	+
Govindan	21	m	rural	russel's	+	+	+	-	+	+	-	-	-
priya	20	f	rural	russel's	-	+	+	-	+	+	+	+	+
Selvakumar	33	m	sub urban	cobra	-	+	+	-	+	+	-	-	-
rahul	19	m	rural	cobra	+	+	+	-	+	+	+	+	-
sengammal	50	f	rural	krait	-	+	-	-	+	+	-	-	-
Bharathan	40	m	rural	cobra	-	+	+	-	+	+	-	-	-
Padmanathan	23	m	sub urban	russel's	+	+	+	-	+	+	-	-	-
Pechiappan	25	m	sub urban	russel's	+	+	+	-	+	+	+	-	-
latha	40	f	sub urban	krait	-	+	-	-	+	+	-	-	-
Vijaykumar	18	m	sub urban	russel's	+	+	+	-	+	+	-	-	-
gowri	35	f	rural	russel's	+	+	+	-	+	+	+	+	-
Lakshmanan	17	m	rural	cobra	-	+	+	-	+	+	-	-	-
amudha	24	f	rural	krait	-	+	-	-	+	+	+	+	+
Rangan	60	m	sub urban	cobra	-	+	+	-	+	+	+	+	+
Paramasivam	21	m	rural	russel's	+	+	+	-	+	+	-	-	-
Prakash	20	m	rural	russel's	-	+	+	-	+	+	+	+	+
Chengiammal	33	f	sub urban	cobra	-	+	+	-	+	+	-	-	-
Karthik	19	m	rural	cobra	+	+	+	-	+	+	+	+	-
Shenbagakumar	50	m	rural	krait	-	+	-	-	+	+	-	-	-
Bharathi	40	f	rural	cobra	-	+	+	-	+	+	-	-	-
Pachiappan	23	m	sub urban	russel's	+	+	+	-	+	+	-	-	-
Chinnakannu	25	m	sub urban	russel's	+	+	+	-	+	+	+	-	-
Arunkumar	16	m	sub urban	krait	-	+	-	-	+	+	-	-	-
vijayalakshmi	18	f	sub urban	russel's	+	+	+	-	+	+	-	-	-
Gokulakannan	35	m	rural	russel's	+	+	+	-	+	+	+	+	-
Narayanan	17	m	rural	cobra	-	+	+	-	+	+	-	-	-
amudha	24	f	rural	krait	-	+	-	-	+	+	+	+	+
Dharshanamoorthy	40	m	sub urban	russel's	+	+	+	-	+	+	-	-	-
Dhandapani	18	m	sub urban	russel's	+	+	+	-	+	+	-	-	-

local	hematuria	bite to asv	asv dose	neostig	surgical	cmap1	velocity 1	snap 1	F wave 1	rns 1	cmap2	velocity 2	snap 2	F wav 2
+	-	3	18	+	+	Dec	n	n	n	Dec	n	N	n	n
+	-	5	18	-	-	Dec	n	n	n	Inc	n	N	n	n
+	-	1	18	-	-	Dec	n	n	n	N	n	n	n	n
+	-	2	18	+	-	Dec	n	n	n	Dec	n	n	n	n
+	-	4	13	-	-	N	n	n	n	N	n	n	n	n
-	-	4	18	-	-	N	n	n	n	Inc	n	n	n	n
+	-	3	13	+	-	Dec	n	n	n	Dec	n	n	n	n
+	-	5	18	-	-	Dec	n	n	n	N	n	n	n	n
+	-	3	18	-	-	N	n	n	n	N	n	n	n	n
-	-	2	18	-	-	Dec	n	n	n	Inc	n	n	n	n
+	-	4	13	-	-	N	n	n	n	N	n	n	n	n
+	-	6	18	-	+	Dec	n	n	n	Inc	n	n	n	n
+	-	3	13	+	-	N	n	n	n	Dec	n	n	n	n
-	-	6	18	-	-	Dec	n	n	n	Inc	n	n	n	n
+	-	3	18	+	+	Dec	n	n	n	Dec	n	n	n	n
+	-	5	18	-	-	Dec	n	n	n	N	n	n	n	n
+	-	1	18	-	-	Dec	n	n	n	N	n	n	n	n
+	-	2	18	+	-	N	n	n	n	N	n	n	n	n
+	-	4	13	-	-	N	n	n	n	Dec	n	n	n	n
-	-	4	18	-	-	N	n	n	n	N	n	n	n	n
+	-	3	13	-	-	N	n	n	n	Inc	n	n	n	n
+	-	5	18	-	-	Dec	n	n	n	N	n	n	n	n
+	-	3	18	-	-	N	n	n	n	N	n	n	n	n
-	-	2	18	-	+	Dec	n	n	n	N	n	n	n	n
+	-	4	13	-	-	N	n	n	n	N	n	n	n	n
+	-	6	18	-	+	N	n	n	n	N	n	n	n	n
+	-	3	13	-	-	N	n	n	n	N	n	n	n	n
-	-	6	18	-	-	Dec	n	n	n	N	n	n	n	n
+	-	2	18	-	+	N	n	n	n	N	n	n	n	n
+	-	4	13	-	-	N	n	n	n	N	n	n	n	n

rns2	urea	creat	wbct1	wbct2	wbct3	duration	ventilatory	dialysis
n	26	0.9	n	n	n	22	+	-
n	70	4.2	hi	hi	hi	7	-	+
n	24	1.2	n	n	n	4	+	-
n	24	0.8	n	n	n	10	-	-
n	18	0.8	hi	n	n	6	-	-
n	18	0.9	n	n	n	3	-	-
n	38	0.7	n	n	n	3	-	-
n	42	1.1	hi	hi	n	5	-	-
n	32	0.8	hi	hi	n	4	-	-
n	24	0.8	hi	hi	n	11	-	-
n	44	1	hi	n	n	3	-	-
n	66	2.8	hi	hi	hi	12	-	-
n	20	0.9	n	n	n	3	-	-
n	28	0.8	n	n	n	12	+	-
n	26	0.9	n	n	n	22	-	-
n	50	1.5	hi	hi	hi	6	-	-
n	24	1.2	n	n	n	4	+	-
n	24	0.8	n	n	n	7	-	-
n	48	2.1	hi	n	n	5	-	-
n	18	0.9	n	n	n	3	-	-
n	38	0.7	n	n	n	3	-	-
n	42	1.1	hi	hi	n	5	-	-
n	32	0.8	hi	hi	n	4	-	-
n	24	0.8	hi	hi	n	11	-	-
n	44	1	hi	n	n	3	-	-
n	53	3.1	hi	hi	hi	12	-	-
n	20	0.9	n	n	n	3	-	-
n	28	0.8	n	n	n	12	+	-
n	24	0.8	hi	hi	n	11	-	-
n	44	1	hi	n	n	3	-	-